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FILE 'HOME' ENTERED AT 06:45:51 ON 20 APR 2001

=> file caplus


=> s cDNA librar?
        116503 CDNA
         54899 LIBRAR?
L1       18100 CDNA LIBRAR?
                 (CDNA(W)LIBRAR?)

=> s l1 (p) (restriction or RFLP)
         72491 RESTRICTION
         11118 RFLP
L2         796 L1 (P) (RESTRICTION OR RFLP)

=> s l2 and clone
         63750 CLONE
L3         371 L2 AND CLONE

=> s l2 and clone?
        168403 CLONE?
L4         657 L2 AND CLONE?

=> s l4 and screen?
        169131 SCREEN?
L5         271 L4 AND SCREEN?

=> s l5 and express?
        713107 EXPRESS?
L6         157 L5 AND EXPRESS?



=> s l1 (p) (restriction fragment or RFLP)
         72491 RESTRICTION
        138906 FRAGMENT
         17297 RESTRICTION FRAGMENT
                 (RESTRICTION(W)FRAGMENT)
         11118 RFLP
L7         220 L1 (P) (RESTRICTION FRAGMENT OR RFLP)

=> s l7 and clone
         63750 CLONE
L8         109 L7 AND CLONE

=> s l8 and screen?
        169131 SCREEN?
L9          46 L8 AND SCREEN?

=> s l9 and express?
        713107 EXPRESS?
L10         28 L9 AND EXPRESS?

=> d abs 1-5

L10  ANSWER 1 OF 28  CAPLUS  COPYRIGHT 2001 ACS
AB   Transcriptomes of the developing seeds of high-oleate and high-linoleate
     strains of safflower (Carthamus tinctorius) were compared using a
     fluorescent differential display technique.  Two cDNA fragments (O1-1 and
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O2-3) were identified as the bands originated from the genes highly or specifically ***expressed*** in the developing seeds of the high-oleate safflower. A cDNA ***clone*** , CTOS-1, encoding a novel protein with an isoprenoid binding site at the C-terminus was isolated by ***screening*** a ***cDNA*** ***library*** constructed from t developing seeds of high-oleate safflower using the O2-3 fragment as a probe. Northern blot anal. indicated that CTOS-1 gene ***expresses*** only in the developing seeds of the high-oleate safflower. The transcrip cannot be detected either in the developing seeds of the high-linoleate safflower nor in the leaves, stems and flower buds of the two strains. Southern blot anal. using the CTOS-1 cDNA as a probe indicated the presence of ***restriction*** ***fragment*** length polymorphism between the high-oleate and high-linoleate strains.

L10 ANSWER 2 OF 28 CAPLUS COPYRIGHT 2001 ACS

AB The eukaryotic translation initiation factor eIF-4A is an ATP-dependent RNA helicase involved in ribosome attachment to the 5 end of mRNAs. Employing as a probe a Cryptosporidium parvum genomic amplicon encoding a partial polypeptide related to eIF-4A, we ***screened*** a C. parvum sporozoite ***cDNA*** ***library*** to ***clone*** the full length of the gene. Two complete cDNAs were characterized, Cp.F6 and Cp.F10, which consisted of 1,900 and 1,418 bp, resp. The overlapping portions of the sequences shared 100% identity and encoded a polypeptide of 405 amino acids whose identity to known eIF-4A mols. ranged between 77 and 39%. The 2 cDNAs differed in the length of their resp. 3′ untranslated regions, of 577 bp in Cp.F6 and 72 bp in Cp.F10, in both of which a putative polyadenylation signal was identified. The structure of the cloned cDNAs, along with genomic Southern blot data indicating that eIF-4A is encoded by a single copy gene, strongly suggested that Cp.F6 an Cp.F10 reflect a differential 3′ end processing of mRNA precursors, not obsd. so far in C. parvum. Northern blot anal. confirmed that the sporozoites ***express*** 2 eIF-4A mRNAs and showed that the lower mol. wt. transcript is 10- to 20- fold more abundant. We also investigated the polymorphism of the eIF-4A gene and defined a novel polymerase chain reaction- ***restriction*** ***fragment*** length polymorphism marker discriminating between C. parvum isolates of genotype 1 and 2.

L10 ANSWER 3 OF 28 CAPLUS COPYRIGHT 2001 ACS

AB Degenerate polymerase chain reaction (PCR) primers based on conserved residues from alignments of species with already characterized major histocompatibility complex (MHC)-encoded sequences were used in the searc for class I and .beta.2-microglobulin (b2m) genes in Atlantic cod (Gadus morhua L.). After PCR amplification and subsequent sequencing a putative class I sequence was identified, from which a probe was designed and used to ***screen*** a spleen ***cDNA*** ***library*** from one single individual. The full-length ***clone*** obtained was sequence and shown to be a classical Mhc class I-encoded sequence. It revealed th characteristic .alpha.1-, .alpha.2-, and .alpha.3-domains and transmembrane and cytoplasmic region, with several conserved amino acids. A PCR amplification from the .alpha.2-domain to the CY-region was performed on the same library, using a proof-reading enzyme. At least 11 unique addnl. sequences were isolated. Moreover, sequencing of the addnl cDNA clones resulted in a total of 17 different Mhc class I sequences in this individual. A Southern hybridization of DNA from four different individuals using an .alpha.3-specific probe confirmed this large no. of genes. Interestingly, based on differences mainly in their transmembrane region, the sequences obtained could be divided into two distinct groups. Within the groups no support could be obtained for any further subdivision. Southern expts. using an .alpha.1-specific probe gave almos the same ***restriction*** ***fragment*** length polymorphism wit a high no. of hybridizing bands, suggesting a low divergence in this part of the gene. Sequencing of PCR clones obtained with a proof-reading

enzyme confirmed this at the nucleotide level. PCR amplification to isolate and characterize the b2m gene resulted in a sequence which was used to ***screen*** a thymus ***cDNA*** ***library*** . Two different alleles were obtained and these showed the characteristic features of known teleostean .beta.2m sequences. A Southern hybridizatio with genomic DNA from four different individuals suggested the presence o one b2m locus in Atlantic cod.

L10 ANSWER 4 OF 28 CAPLUS COPYRIGHT 2001 ACS
AB The Bundle sheath defective2 (Bsd2) gene is required for ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) accumulation in maize. Using a Mutator transposable element as a mol. probe, we identified a tightly linked ***restriction*** ***fragment*** length polymorphism that cosegregated with the bsd2-conferred phenotype. This fragment was cloned, and sequences flanking the Mutator insertion were used to ***screen*** a maize leaf ***cDNA*** ***library*** . Using a full-length cDNA ***clone*** isolated in this ***screen*** , we show that an abundant 0.6-kb transcript could be detected in wild-type plants but not in bsd2-mL plants. This 0.6-kb transcript accumulated to low levels in plants carrying an allele derived from bsd2-mL that conditions a less severe mutant phenotype. Taken together, these data strongly suggest that we have cloned the Bsd2 gene. Sequence anal. of the full-length cDNA ***clone*** revealed a chloroplast targeting sequence and a region of homol. shared between BSD2 and the DnaJ class of mol. chaperones. This region of homol. is limited to a cysteine-rich Zn binding domain in DnaJ believed to play a role in protein-protein interactions. We show that BSD2 is targeted to the chloroplast but is not involved in general photosynthetic complex assembl or protein import. In bsd2 mutants, we could not detect the Rubisco protein, but the chloroplast-encoded Rubisco large subunit transcript (rbcL) was abundant and assocd. with polysomes in both bundle sheath and mesophyll cells. By characterizing Bsd2 ***expression*** patterns an analyzing the bsd2-conferred phenotype, we propose a model for BSD2 in th post-translational regulation of rbcL in maize.

L10 ANSWER 5 OF 28 CAPLUS COPYRIGHT 2001 ACS
AB Membrane-assocd. proteins were isolated from adult Philippine strain S. japonicum by partitioning into the detergent phase of Triton X-114. A rabbit polyclonal antiserum raised against these proteins was used to ***screen*** an S. japonicum ***expression*** ***cDNA*** ***library*** . Pos. clones were identified which encoded the species orthologue of SmIrV1, a Schistosoma mansoni protein which was initially identified by ***screening*** with sera from mice protectively vaccinated with irradiated cercariae. The S. japonicum mol., which we term SjIrV1, is 83% identical to SmIrV1 at the predicted amino acid level and is a member of the calreticulin family of non-EF-hand, calcium-bindin proteins. The Chinese strain S. japonicum orthologue of SjIrV1 was obtained by ***screening*** with the radiolabeled insert of the Philippine strain ***clone*** . Northern blot anal. revealed a single message of around 2.4 kb and gave no indication of alternative splicing. Southern blot anal. gave a simple pattern, indicating a single-copy gene, and showed a single ***restriction*** ***fragment*** length polymorphism between the genomes of Chinese and Philippine strains of S. japonicum. Recombinant, full-length SjIrV1 was ***expressed*** with hexahistidine tag in Escherichia coli and the recombinant protein isolate by nickel-chelate chromatog. Recombinant SjIrV1 was shown to exhibit calcium-dependent, differential electrophoretic migration and to bind ruthenium red in the absence but not in the presence of calcium ions. Th presence of conserved Ca2+-binding motifs predicted from the primary sequence, together with the Ca2--dependent electrophoretic mobility of recombinant SjIrV1, confirmed that SjIrV1 was a functional calcium-bindin protein.